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Estimation of Minimum Whole-Blood Tacrolimus Concentration for Therapeutic Drug Monitoring with Plasma Prednisolone Concentration: A Retrospective Cohort Study in Japanese Kidney Transplant Recipients

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ABSTRACT

Background: In immunosuppressive therapy administered after organ transplantation, therapeutic drug monitoring (TDM) of tacrolimus must be performed frequently because of the large variation in its pharmacokinetic properties and a progressive decrease in dose requirements. An indicator for estimating the target minimum whole-blood tacrolimus concentration ($C_{min\ TAC}$) would be useful to minimize the number of blood samplings required for tacrolimus TDM.

Objectives: The primary objective of this study was to investigate whether plasma prednisolone concentration, postoperative days (POD) and AUC 0 to 9 hours before transplantation (AUC_{0-9int}) are useful indicators of tacrolimus TDM. The secondary objective was to determine the usefulness of blood tacrolimus concentration as an indicator of the development of nontraumatic, glucocorticoid-induced necrosis of the femoral head, an adverse event that has been associated with the use of prednisolone in vivo.

Methods: This open-label, nonrandomized, retrospective study was conducted at the Department of Transplantation and Regenerative Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan. Data from 43 male and 22 female patients (mean age, 38 years [range, 9–64 years]) who received a living-related kidney transplant from 2001 to 2004 were included. Multiple blood samplings were performed to determine AUC_{0-9int}, AUC 0 to 9 hours after drug administration and after transplantation (AUC_{0-9}), $C_{min\ TAC}$, C_{max}, and T_{max} after transplantation. The correlations between each parameter were determined. The correlation between POD and the changes in tacrolimus bioavailability was investigated

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using the indicator, defined as the tacrolimus dose required to maintain the target (10–15 ng/mL) $C_{min\ TAC}$ (dose/$C_{10–15}$). Correlations between dose/$C_{10–15}$ and $AUC_{0–9int}$ (3 $AUC_{0–9int}$ groups, defined as follows: low, medium, and high [<93, ≥93–<152, and ≥152 ng · h/mL, respectively]) were determined. Correlations between mean C_{min} values of prednisolone at a dose of 40 mg on PODs 4 to 11 ($C_{min\ PSL40}$) and $C_{min\ TAC}$, or $AUC_{0–9int}$ were determined. A subanalysis was used to determine the relationship between dose/$C_{10–15}$ and the prevalence of nontraumatic, glucocorticoid-induced necrosis of the femoral head.

Results: $C_{min\ TAC}$ was found to be significantly correlated with $AUC_{0–9int}$ (r = 0.554; $P < 0.001$) and $C_{min\ PSL40}$ (r = 0.336; $P < 0.001$). In the low-$AUC_{0–9int}$ group, dose/$C_{10–15}$ was higher than that of the other groups ($P < 0.01$). $AUC_{0–9int}$ was significantly correlated with $C_{min\ PSL40}$ (r = 0.445; $P < 0.001$). Dose/$C_{10–15}$ in the patient group that had necrosis of the femoral head was lower than that of the group without necrosis (n = 6; $P < 0.01$).

Conclusions: The results of this small, retrospective study suggest that $C_{min\ PSL40}$, $AUC_{0–9int}$, and POD were significant predictors of $C_{min\ TAC}$. These parameters were found to be a useful indicator of tacrolimus TDM in these Japanese transplant recipients. Our results also suggest that dose/$C_{10–15}$ and $AUC_{0–9int}$ might be useful indicators for estimating the risk for nontraumatic, steroid-induced necrosis of the femoral head. (*Curr Ther Res Clin Exp.* 2006;67: 103–117)

Key words: tacrolimus, prednisolone, blood concentration, therapeutic drug monitoring.

INTRODUCTION

Tacrolimus, a macrolide lactone, is an immunosuppressive agent that inhibits the signal-transduction pathway that leads to T-lymphocyte activation.[1–3] Tacrolimus has been used for preventing or treating graft rejection after organ transplantation.[4–6] Therapeutic drug monitoring (TDM) is essential to minimizing toxicity while maintaining the efficacy of immunosuppressants such as tacrolimus, which has high variability in its pharmacokinetic (PK) properties.[7–9] In the TDM of tacrolimus, the most widely used indicator is minimum whole-blood tacrolimus concentration ($C_{min\ TAC}$).[10] To maintain target $C_{min\ TAC}$, TDM must be performed frequently, every day until 14 postoperative days (PODs) in our institution.[11] To minimize the number of blood samplings required for tacrolimus TDM, an indicator for estimating $C_{min\ TAC}$ would be useful. In general, AUC is a useful indicator for TDM because systemic exposure is measured as AUC. Therefore, to establish the possible usefulness of a method for estimating $C_{min\ TAC}$, we investigated the PK properties of tacrolimus in kidney transplant recipients, including AUC 0 to 9 hours after drug administration before transplantation ($AUC_{0–9int}$) and 0 to 9 hours after drug administration and after transplantation ($AUC_{0–9}$).

Previous studies have reported a progressive decrease in tacrolimus dose requirements as time after adult organ transplant recipients elapsed.[12,13] How-

ever, in Japanese kidney transplant recipients, time-dependent increases in the bioavailability of tacrolimus within 4 weeks of transplantation were not found in a MEDLINE search of the literature (key terms: *tacrolimus*, *kidney [renal]*, *pharmacokinetics*, *bioavailability [AUC]*, and *time after transplantation [time-dependent]*; years: 1974–2006). If time dependent increase of blood clearance was observed in Japanese kidney transplant patients, POD might be another useful parameter for estimating $C_{min\ TAC}$.

Prednisolone, a synthetic glucocorticoid, is commonly used in the treatment of a variety of inflammatory diseases such as rheumatism, hepatitis, and nephritis. After kidney transplantation, prednisolone is used concomitantly with tacrolimus or cyclosporin A in immunosuppressive therapy in all cases of kidney transplantation in our institution.[14] Adverse events (AEs) associated with the use of prednisolone include nontraumatic, glucocorticoid-induced necrosis of the femoral head. About 6% of kidney transplant recipient's nontraumatic, glucocorticoid-induced necrosis of the femoral head was developed in our institution.[15] Despite using the same protocol for prednisolone administration, studies in adult kidney transplant recipients have found different clinical courses of this disorder, indicating individual differences in steroid sensitivity.[16]

Prednisolone and tacrolimus are metabolized by the same isoenzyme—cytochrome P450 (CYP) 3A4.[17,18] Competitive inhibition has been reported with combination treatment with these 2 drugs.[18] Both drugs are a substrate of P-glycoprotein, a transporter that plays an important role in drug absorption and distribution. Therefore, it is hypothesized that tacrolimus and prednisolone have a similar absorption and clearance rate in the body. In addition, the bioavailability of prednisolone is almost 100%,[19–21] with the result that plasma prednisolone concentrations per dose reflect prednisolone clearance. Prednisolone is typically administered as a fixed-dose regimen in transplant recipients in our institution.[14] For this reason, and because tacrolimus has the same elimination pathway and large variations in bioavailability, we hypothesized that plasma prednisolone concentrations might serve as an indicator of tacrolimus TDM in patients receiving immunosuppressive regimens after transplantation, and that the risks for AEs associated with prednisolone use can be estimated using tacrolimus TDM.

The primary objective of this study was to investigate whether plasma prednisolone concentration is a useful indicator of tacrolimus TDM. The secondary objective was to determine whether $C_{min\ TAC}$ is useful as an indicator of the development of nontraumatic, glucocorticoid-induced necrosis of the femoral head.

PATIENTS AND METHODS

This open-label, nonrandomized, retrospective cohort study was conducted at the Department of Transplantation and Regenerative Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan. The study protocol was approved by the

ethics committee at the university, and informed consent was obtained from all patients or their legal guardians (for patients aged <18 years).

Study Population

Data from the medical records of Japanese patients (43 male and 22 female; mean age, 38 years [range, 9–64 years]) who had received a living-related kidney transplant at the university from 2001 to 2004 were included. All patients who had been administered tacrolimus treatment as described earlier were retrospectively enrolled in the study.

To investigate the relationship between the PK properties of tacrolimus and plasma prednisolone concentration, the patients for whom these data were available were included in this subanalysis.

Immunosuppressive Regimen

The following immunosuppressive regimen, developed by the Department of Transplantation and Regenerative Surgery for use after living-related kidney transplantation, was used in the patients. The initial dose of tacrolimus (0.3 mg · kg^{-1} · d^{-1}) was administered orally for 2 days before transplantation. Tacrolimus (0.1 mg · kg^{-1} · d^{-1}) was administered by continuous IV infusion on the day of transplantation before surgery, followed by oral tacrolimus (0.3 mg/kg BID) for 3 weeks. The dose of tacrolimus was adjusted based on the results of $C_{min\ TAC}$ monitoring empirically. Target $C_{min\ TAC}$ was 10 to 15 ng/mL. Methylprednisolone 500 mg was administered by continuous IV infusion on the day of transplantation before surgery, followed by prednisolone 50 mg/d on PODs 0 to 3 and prednisolone 40 mg/d on PODs 4 to 11. On PODs 12 to 19, the dose of prednisolone was decreased to 30 mg/d, and subsequently reduced by 5 mg each week until a dose of 10 mg/d was reached. On POD 21, azathioprine (0.50–0.75 mg/kg PO BID) or mycophenolate mofetil (10.0–12.5 mg/kg PO BID) was added to the regimen. This was continued until the patient was discharged from the hospital.

Pharmacokinetic Assessment of Tacrolimus

For PK studies of tacrolimus, blood samples were obtained 2 days before transplantation and at 0, 1, 2, 3, 4, 6, and 9 hours after drug administration on PODs 14, 21, and 28. PK parameters (eg, AUC_{0-9}, C_{max}, T_{max}) were determined from those data. To determine $C_{min\ TAC}$, blood samples were obtained before drug administration each day through POD 14. After POD 14, blood samples were obtained before drug administration every other day until POD 28. Blood tacrolimus concentration was measured using microparticle enzyme immunoassay (IMx system, Abbott Laboratories, Abbott Park, Illinois).[22] AUC_{0-9} and AUC_{0-9int} were calculated using the trapezoidal rule.

Plasma Prednisolone Concentration

Plasma prednisolone concentration was measured using the high-performance liquid chromatography (HPLC) method described by Rose and Jusko,[23] of which

internal standard, the mobile phase, and organic sorbent for liquid-liquid extraction were modified by us. Plasma samples for the determination of prednisolone concentration were collected before oral administration of prednisolone 40 mg/d on PODs 4 to 11. More than 3 blood samples (2 mL) were obtained between PODs 4 to 11 from each patient, and the mean prednisolone concentration was calculated and considered as an indicator of prednisolone clearance at an early stage of prednisolone treatment (C_{min} values of prednisolone at dose of 40 mg on PODs 4–11 [$C_{min\ PSL40}$]). The prednisolone and carbamazepine used as internal standards were purchased from Nacalai Tesque, Kyoto, Japan. Acetonitrile and methanol were HPLC grade; all other reagents were reagent grade. The preparation of plasma samples was based on liquid-liquid extraction with dichloromethane. The compounds were separated on a cyanopropyl column using acetonitrile-methanol-water (66:17:17, v/v) as the mobile phase. UV detection was used at a wavelength of 254 nm. The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of an LC-10A liquid delivery module, an SPD-10A UV detector, a CTO-10A column oven, and a Simpac CLC-CN column (150 mm × 6 mm; interior diameter, 5 μm). Samples were injected using an SIL-10A automatic injector. The system was controlled with an SCL-10A system controller. The area under each objective peaks in chromatograph were calibrated with a Shimadzu CR-8A data processor. The standard curves of prednisolone ranging from 0.01 to 500 μg/mL were considered linear. CV values (%) of inter- and intra-assay reproducibility were <10%.

Tacrolimus Concentration as an Indicator of Prednisolone Adverse Events

To investigate whether blood tacrolimus concentration might be useful as an indicator of the development of AEs associated with prednisolone use, the relationship between tacrolimus bioavailability in patients with and without nontraumatic, glucocorticoid-induced necrosis of the femoral head was investigated using the tacrolimus dose required to maintain the target (10–15 ng/mL) $C_{min\ TAC}$ (dose/$C_{10–15}$) as the indicator of bioavailability. This subanalysis used data from patients diagnosed with nontraumatic, steroid-induced necrosis of the femoral head (diagnosed according to the criteria of the Research Committee of the Ministry of Health and Welfare of Japan[24]); data from the remaining patients were used as references.

Statistical Analysis

The correlations between $C_{min\ TAC}$ or $AUC_{0–9}$ and tacrolimus dose, C_{max}, or T_{max} were determined. After POD 14, dose/$C_{10–15}$ was considered an indicator of bioavailability. The relationship between POD and dose/$C_{10–15}$ was investigated. The correlations between dose/$C_{10–15}$, POD, and $AUC_{0–9int}$ were determined using the following method. Study patients were assigned to 1 of 3 $AUC_{0–9int}$ groups: low, medium, and high (<93, ≥93–<152, and ≥152 ng · h/mL, respectively). On days 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, 31 to 35, and 36 to 40, differences in mean dose/$C_{10–15}$ in each period between the 3 $AUC_{0–9int}$

groups were examined using 1-way analysis of variance. To investigate whether POD, AUC_{0-9int}, and/or plasma prednisolone concentrations are useful as an indicator of tacrolimus TDM, the correlations between $C_{min\ TAC}$, POD, tacrolimus dose, prednisolone dose, AUC_{0-9int}, and $C_{min\ PSL40}$ were determined using Pearson correlation. To assess the relative effects of each parameter on $C_{min\ TAC}$, stepwise multiple linear regressions were performed using POD, tacrolimus dose, prednisolone dose, $C_{min\ PSL40}$, and AUC_{0-9int} as independent variables.

Differences in dose/C_{10-15} between the groups with and without nontraumatic, steroid-induced necrosis of the femoral head for 5 periods of time (PODs 16–20, 21–25, 26–30, 31–35, and 36–40) were determined using the Student *t* test.

Analyses in this study were conducted using the StatView software package version 5.0 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Pharmacokinetic Properties of Tacrolimus

The PK analysis of tacrolimus included 13 patients from whom these data were available (7 males, 6 females; range, 9–62 years).

Figure 1 shows AUC_{0-9} of tacrolimus at various doses on PODs 14, 21, and 28. Although there was a large variation in the dose of tacrolimus (0.15–0.30 mg/kg), no significant differences in mean AUC_{0-9} were found between the 3 time points. Similarly, no significant differences in mean AUC_{0-9} were found between mean doses (0.30, 0.25, 0.20, and 0.15 mg/kg) on PODs 14, 21, and 28.

Table I shows the correlations between the values of AUC_{0-9} and $C_{min\ TAC}$ for C_{max}, T_{max}, amount (mg), and dose (mg/kg) on PODs 14, 21, and 28. At all time points, AUC_{0-9} was significantly correlated with C_{max} (r = 0.829, 0.925, and 0.904, respectively; all, $P < 0.001$), and $C_{min\ TAC}$ was significantly correlated with AUC_{0-9} (r = 0.881, 0.822, and 0.886, respectively; all, $P < 0.001$) and C_{max} (r = 0.636, 0.621, and 0.797, respectively; $P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively). Neither AUC_{0-9} nor $C_{min\ TAC}$ was significantly associated with values of T_{max}, amount, or dose.

The relationship between POD and dose/C_{10-15} as an indicator of bioavailability on POD 14 to 40 is shown in **Figure 2**. As time after transplantation increased, the dose/C_{10-15} decreased gradually (r = –0.830; $P < 0.001$).

Figure 3 shows the relationship between mean dose/C_{10-15} and AUC_{0-9int}. In all periods (PODs 16–20, 21–25, 26–30, 31–35, and 36–40), in the low-AUC_{0-9int} group, mean dose/C_{10-15} was higher (≥0.253 mg · kg^{-1} · d^{-1}) than that in the other groups (≥0.155 mg · kg^{-1} · d^{-1}), and in the middle-AUC_{0-9int} group, mean dose/C_{10-15} was higher (≥0.199 mg · kg^{-1} · d^{-1}) than that in the high-AUC_{0-9int} group (≥0.155 mg · kg^{-1} · d^{-1}). Statistically significant differences in tacrolimus dose/C_{10-15} between all 3 groups in all periods were observed (all, $P < 0.01$). In each group, mean dose/C_{10-15} gradually decreased with POD (low [r = –0.927; $P < 0.01$]; middle [r = –0.960; $P < 0.005$]; high [r = –0.903; $P < 0.05$]).

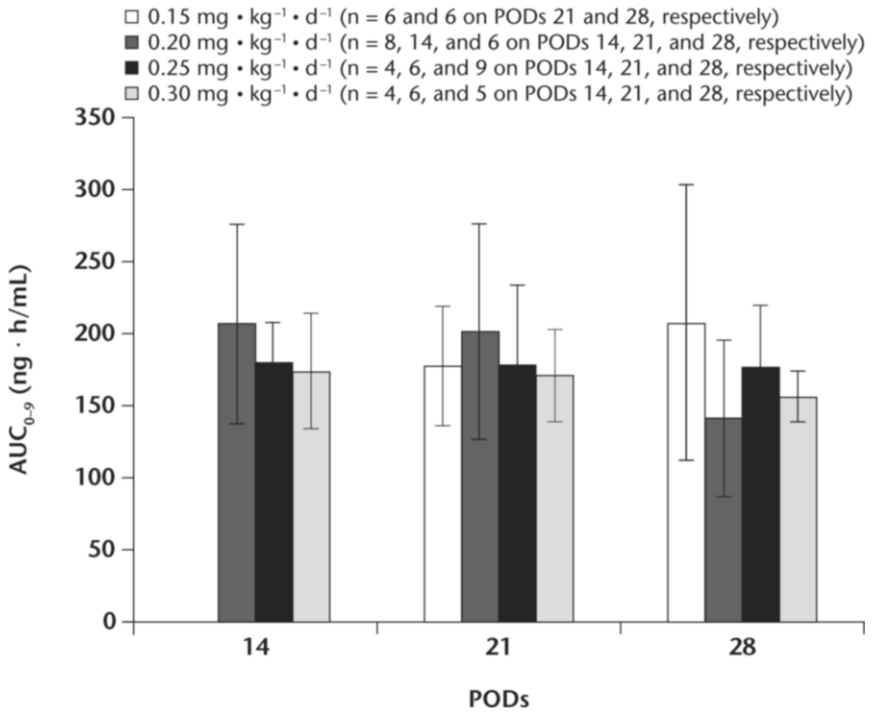

Figure 1. Mean (SD) AUC 0 to 9 hours ($AUC_{0–9}$) after administration of various doses of tacrolimus on postoperative days (PODs) 14, 21, and 28 in Japanese kidney transplant recipients (N = 65). No significant between-group differences were found. Data were unavailable for the 0.15–mg · kg^{-1} · d^{-1} dose at POD 14.

Relationship Between Plasma Prednisolone Concentration and Tacrolimus Pharmacokinetic Properties

$C_{min\ PSL40}$ obtained from the 13 patients from whom data were available varied from 19.5 to 161.4 ng/mL (mean [SD], 56.1 [42.3] ng/mL). The correlations between $C_{min\ TAC}$ and POD; tacrolimus dose, prednisolone dose, $AUC_{0–9int}$, and $C_{min\ PSL40}$; and between $C_{min\ PSL40}$ and $AUC_{0–9int}$ are shown in **Table II**. $C_{min\ TAC}$ was significantly negatively correlated with POD ($r = –0.327$; $P < 0.001$) and significantly positively correlated with $AUC_{0–9int}$ ($r = 0.554$) and $C_{min\ PSL40}$ ($r = 0.336$) (both, $P < 0.001$). $C_{min\ TAC}$ was not significantly associated with the tacrolimus or prednisolone dose. $C_{min\ PSL40}$ was significantly correlated with $AUC_{0–9int}$ ($r = 0.445$; $P < 0.001$). $C_{min\ TAC}$ was used as a dependent variable in stepwise multiple linear regressions that were performed. Four variables (POD, prednisolone dose, $C_{min\ PSL40}$, and $AUC_{0–9int}$) were selected and entered into the regression equation (**Table III**).

Table I. Relationships between the pharmacokinetic properties of tacrolimus in Japanese kidney transplant recipients. Values are correlation coefficients.

Time Point	AUC_{0-9}	C_{max}	T_{max}	Amount	Dose
POD 14 (n = 16)					
AUC_{0-9}	–	0.829*	0.053	0.107	–0.281
$C_{min\ TAC}$	0.881*	0.636†	0.304	0.072	–0.151
POD 21 (n = 32)					
AUC_{0-9}	–	0.925*	0.100	0.068	–0.006
$C_{min\ TAC}$	0.822*	0.621†	–0.049	0.091	–0.251
POD 28 (n = 26)					
AUC_{0-9}	–	0.904*	0.320	0.122	–0.139
$C_{min\ TAC}$	0.886*	0.797*	–0.104	0.196	–0.204

AUC_{0-9} = AUC 0 to 9 hours after drug administration and after transplantation; POD = postoperative day; $C_{min\ TAC}$ = minimum whole-blood tacrolimus concentration.
*$P < 0.001$.
†$P < 0.01$.

Relationship Between Dose/C_{10-15} and Nontraumatic, Steroid-Induced Necrosis of the Femoral Head

The subanalysis of necrosis included 6 patients (4 males, 2 females; mean age, 33 years [range, 21–47 years]) with a diagnosis of nontraumatic, steroid-induced necrosis of the femoral head; data from the remaining 59 patients were used as references.

Figure 4 shows the comparison of mean dose/C_{10-15} between the groups with and without nontraumatic, steroid-induced necrosis of the femoral head on PODs 16 to 20, 21 to 25, 26 to 30, 31 to 35, and 36 to 40. In each period, the mean dose/C_{10-15} of the group that had steroid-induced necrosis of the femoral head was higher (≥0.189 mg · kg^{-1} · d^{-1}) than that of the group that did not have one (≥0.122 mg · kg^{-1} · d^{-1}). Statistically significant differences were observed between the 2 groups in each period (all, $P < 0.01$). In both groups, mean dose/C_{10-15} gradually decreased with POD.

DISCUSSION

Tacrolimus treatment requires regular TDM because of its narrow therapeutic range of blood concentration and large variability in PK properties. A strong correlation between systemic exposure, AUC, and $C_{min\ TAC}$ has been reported in patients who have undergone liver, kidney, and bone marrow transplantation.[25] $C_{min\ TAC}$ was significantly correlated with AUC_{0-9} (r = 0.822–0.886) in this study, suggesting that $C_{min\ TAC}$ was a good indicator for tacrolimus TDM, reflecting a pharmacologic efficacy. On the other hand, AUC_{0-9} was not found to be signifi-

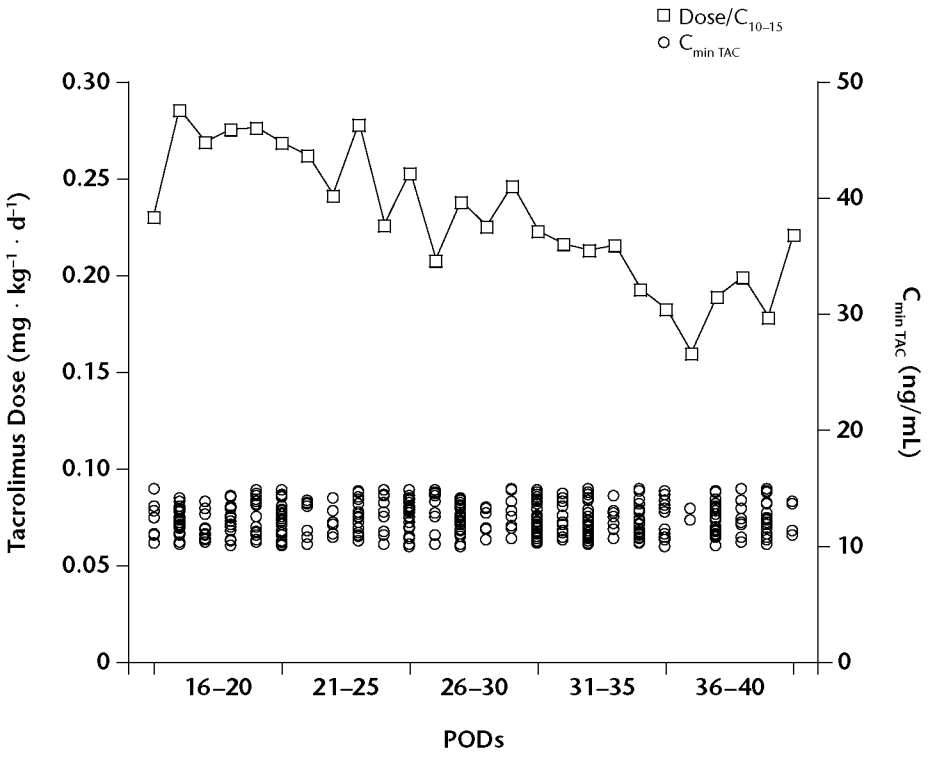

Figure 2. Changes in tacrolimus dose required to maintain target (10–15 ng/mL) minimum whole-blood tacrolimus concentration ($C_{min\ TAC}$) (dose/$C_{10–15}$) as an indicator of bioavailability after postoperative day (POD) 14 in Japanese kidney transplant recipients (N = 65).

cantly correlated with amount or dose. Consequently, a patient's weight might not be a useful indicator of dose requirements.

The bioavailability of tacrolimus has large inter- and intravariability (20%–80%).[25,26] Nonlinear blood binding and a variable blood/plasma ratio have been reported to be major sources of interindividual variations in the PK properties of tacrolimus.[25] Also, tacrolimus is a substrate of P-glycoprotein and CYP 3A4. In both proteins, the induction or inhibition and the change in activation caused by drug interactions are well known. Relationships between the polymorphisms of the *CYP 3A4/5* or *multidrug resistance-1* gene and the tacrolimus required dose to achieve adequate blood concentration have also been found.[27] In this study, although the tacrolimus dose varied substantially (0.15–0.30 mg/kg), no significant changes in mean $AUC_{0–9}$ were associated with the level of $C_{min\ TAC}$. Subsequently, tacrolimus TDM must be performed frequently.[28]

In this study, to obtain useful information for revising the dosing regimen after kidney transplantation, we investigated the change in the bioavailabil-

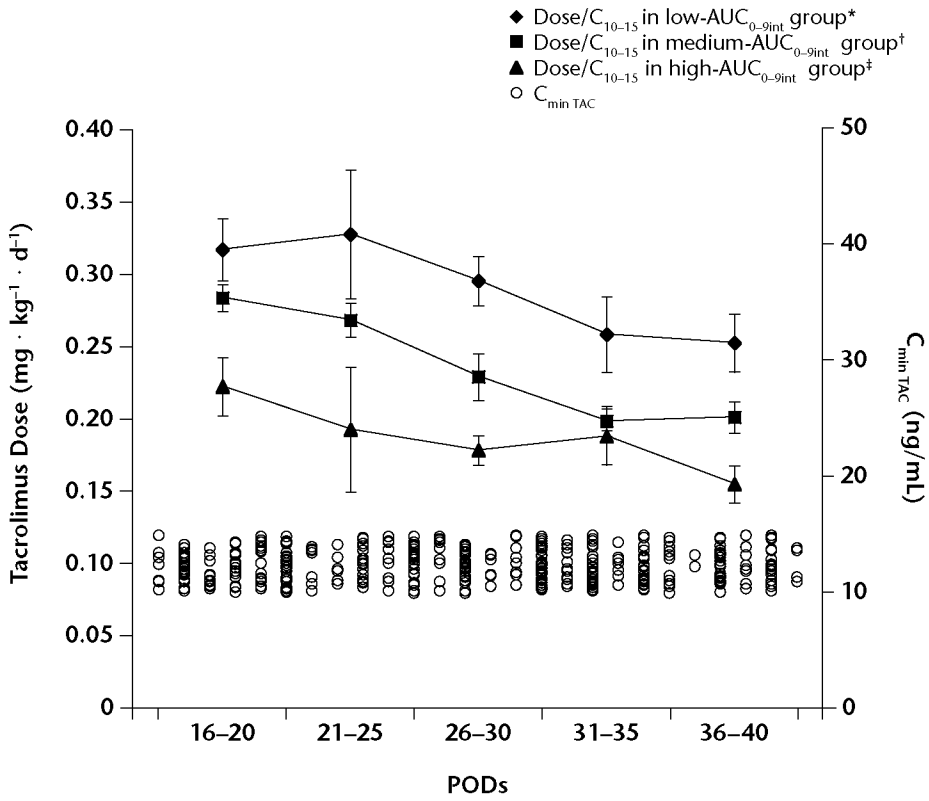

Figure 3. Mean (SD) changes in tacrolimus dose required to maintain target (10–15 ng/mL) minimum whole-blood tacrolimus concentration ($C_{min\ TAC}$) (dose/$C_{10–15}$) as an indicator of bioavailability after postoperative day (POD) 14 in Japanese kidney transplant recipients (N = 65), by preoperative AUC ($AUC_{0–9int}$). $P < 0.01$ between all groups at all time points. *$AUC_{0–9int}$ <93 ng · h/mL; †$AUC_{0–9int}$ ≥93–<152 ng · h/mL; ‡$AUC_{0–9int}$ ≥152 ng · h/mL.

Table II. Relationships between minimum whole-blood tacrolimus concentration ($C_{min\ TAC}$) or minimum plasma concentration with prednisolone 40 mg ($C_{min\ PSL40}$) on postoperative days (PODs) 4 to 11 and other pharmacokinetic parameters in Japanese kidney transplant recipients (N = 65 for $C_{min\ TAC}$ and N = 13 for $C_{min\ PSL40}$). Values are correlation coefficients.

Parameter	POD	Tacrolimus Dose	Prednisolone Dose	$AUC_{0–9int}$	$C_{min\ PSL40}$
$C_{min\ TAC}$	–0.327*	0.042	0.212	0.554*	0.336*
$C_{min\ PSL40}$	–	–	–	0.445*	–

$AUC_{0–9int}$ = AUC 0 to 9 hours before transplantation.
*$P < 0.001$.

Table III. Multiple linear regression model for minimum whole-blood tacrolimus concentration ($C_{min\ TAC}$) in Japanese kidney transplant recipients.*

Dependent Variable	Independent Variables	Correlation Coefficient
$C_{min\ TAC}$	POD	–0.352
	Prednisolone dose	–0.226
	$C_{min\ PSL40}$	0.102
	AUC_{0-9int}	0.048
	Intercept	20.408

POD = postoperative day; $C_{min\ PSL40}$ = minimum plasma concentration with prednisolone 40 mg (days 4–11); AUC_{0-9int} = AUC 0 to 9 hours before transplantation.
*Multiple regression coefficient: $r = 0.676$; $P < 0.001$ (F test).

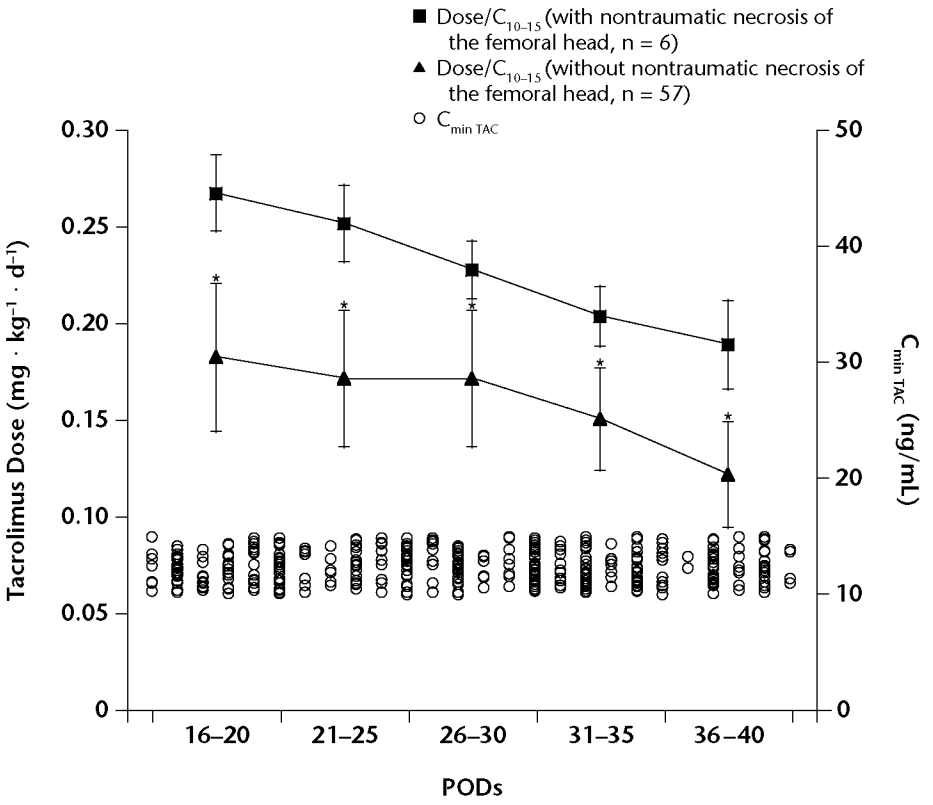

Figure 4. Mean (SD) changes in tacrolimus dose required to maintain target (10–15 ng/mL) minimum whole-blood tacrolimus concentration ($C_{min\ TAC}$) (dose/C_{10-15}) as an indicator of bioavailability after postoperative day (POD) 14 in Japanese kidney transplant patients with (n = 6) or without (n = 59) nontraumatic, steroid-induced necrosis of the femoral head. *$P < 0.01$ between groups.

ity of tacrolimus in the course of immunosuppressive therapy. After transplantation, mean dose/$C_{10–15}$ decreased (ie, bioavailability increased). Moreover, a significant negative correlation between $C_{min\ TAC}$ and POD ($r = –0.327$; $P < 0.001$) was found. These results suggest that POD is a good indicator for estimating $C_{min\ TAC}$. The reasons hypothesized for increased bioavailability were as follows: (1) tacrolimus absorption increased with the recovery of gastrointestinal function; (2) bile secretion increased with increased food intake; and (3) clearance of tacrolimus decreased with increasing serum protein-binding ratio because of increasing serum albumin concentration with the recovery of kidney function.

$AUC_{0–9int}$ is useful for individualizing dosing regimens before transplantation. In the low-$AUC_{0–9int}$ group, mean dose/$C_{10–15}$ was higher than that in the other groups in all periods after transplantation, suggesting that patients with low $AUC_{0–9int}$ needed higher doses of tacrolimus compared with patients in the high- or middle-$AUC_{0–9int}$ group. In addition, $C_{min\ TAC}$ was positively correlated with $AUC_{0–9int}$ ($r = 0.554$; $P < 0.001$), suggesting that the important factors for estimating $C_{min\ TAC}$ were POD and $AUC_{0–9int}$.

On the other hand, it is possible that tacrolimus has PK properties similar to those of prednisolone in vivo, as previously hypothesized. Values of $AUC_{0–9int}$ and $C_{min\ TAC}$ were significantly correlated with $C_{min\ PSL40}$ in this particular study ($r = 0.445$ and 0.336, respectively; both, $P < 0.001$). At Kyoto Prefectural University of Medicine, prednisolone is administered as a fixed regimen at a relatively high dose (40 mg/d) until 11 days after transplantation. We hypothesized that $C_{min\ PSL40}$ would reflect prednisolone clearance and would be a useful indicator of tacrolimus TDM. The results of multiple linear regressions also suggest that the most important factors for estimating $C_{min\ TAC}$ were POD, $AUC_{0–9int}$, and $C_{min\ PSL40}$. TDM of prednisolone using plasma concentration as an indicator was not found to be useful, perhaps because of the unclear relationship between plasma prednisolone concentration and its therapeutic effects. Therefore, plasma prednisolone concentration is not commonly determined. However, our results suggest that the assessment of plasma prednisolone concentration might be a useful indicator of tacrolimus TDM. However, standards need to be established for measuring tacrolimus AUC before transplantation and determining plasma prednisolone concentration at an early stage after transplantation.

Types of nontraumatic necrosis of the femoral head include aseptic and ischemic necrosis. The mechanism by which necrosis develops is unknown, but accumulated statistical data have identified treatment with glucocorticoid, such as prednisolone, as a relevant risk factor. It is notable that the nontraumatic, steroid-induced type of necrosis of the femoral head accounts for a large percentage of cases of this disease. It has been reported that about 60% of nontraumatic necrosis of the femoral head was glucocorticoid-induced.[29] Mean dose/$C_{10–15}$ in the group with necrosis was lower than that in the group without necrosis. It is considered that the bioavailability of tacrolimus was

high in the group with necrosis, suggesting prednisolone clearance might be low after transplantation. Therefore, tacrolimus TDM might be useful for estimating the risk for nontraumatic necrosis of the femoral head. In addition, prednisolone was administered as a fixed-dose regimen, although $C_{min\ PSL40}$ varied substantially (19.5–161.4 ng/mL). Therefore, in determining the risk for nontraumatic necrosis of the femoral head, dose/C_{10-15} of tacrolimus was found to be useful as an indicator of prednisolone clearance. On the other hand, from the beginning of glucocorticoid treatment, nontraumatic necrosis of the femoral head should be prevented.[30]

In this study, AUC_{0-9int} was found to be significantly correlated with $C_{min\ PSL40}$ ($r = 0.445$; $P < 0.001$), reflecting prednisolone clearance in the early post transplantation stage and suggesting that AUC_{0-9int} might be a useful indicator for estimating the risk for steroid-induced necrosis of the femoral head, although the usefulness of this parameter is limited by the need for a measurement of tacrolimus AUC before transplantation. Another limitation of this study was its small sample size. Additional research in a larger population is needed.

CONCLUSIONS

The results of this small, retrospective study suggest that $C_{min\ PSL40}$, AUC_{0-9int}, and POD were significant predictors of $C_{min\ TAC}$. These parameters were found to be a useful indicator of tacrolimus TDM in these Japanese kidney transplant recipients. Our results also suggest that dose/C_{10-15} and AUC_{0-9int} might be useful indicators for estimating the risk for nontraumatic, steroid-induced necrosis of the femoral head.

REFERENCES

1. Kino T, Hatanaka H, Hashimoto M, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. *J Antibiot (Tokyo)*. 1987;40:1249–1255.
2. Kino T, Hatanaka H, Miyata S, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK506 in vitro. *J Antibiot (Tokyo)*. 1987;40:1256–1265.
3. Sigal NH, Dumont FJ. Cyclosporin A FK-506, and rapamycin: Pharmacologic probes of lymphocyte signal transduction. *Annu Rev Immunol*. 1992;10:519–560.
4. Starzl TE, Todo S, Fung J, et al. FK-506 for liver, kidney, and pancreas transplantation. *Lancet*. 1989;2:1000–1004.
5. Uemoto S, Tanaka K, Honda K, et al. Experience with FK506 in living-related liver transplantation. *Transplantation*. 1993;55:288–292.
6. Plosker GL, Foster RH. Tacrolimus. A further update of its pharmacology and therapeutic use in the management of organ transplantation. *Drugs*. 2000;59:323–389.
7. Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation*. 1996;62:920–926.

8. McMaster P, Mirza DF, Ismail T, et al. Therapeutic drug monitoring of tacrolimus in clinical transplantation. *Ther Drug Monit.* 1995;17:602–605.
9. Jusko WJ, Thomson AW, Fung J, et al. Consensus document: Therapeutic monitoring of tacrolimus (FK-506). *Ther Drug Monit.* 1995;17:606–614.
10. Ihara H, Shinkurma D, Ichikawa Y, et al. Intra- and interindividual variation in the pharmacokinetics of tacrolimus (FK506) in the kidney transplant recipients—importance of trough level as a practical indicator. *Int J Urol.* 1995;2:151–155.
11. Okamoto M, Omori Y, Kadotani Y, et al. Tacrolimus-based immunosuppression in renal transplantation: 78 Experiences in a single Japanese center. *Transplant Proc.* 2002;34:1666.
12. Staatz CE, Willis C, Taylor PJ, et al. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. *Clin Pharmacol Ther.* 2002;72:660–669.
13. Okamoto M, Yoshimura N, Nakai I, et al. Beneficial effect of multiple drug therapy including tacrolimus in clinical renal transplantation. *Transplant Proc.* 2000;32:1716–1717.
14. Yasumura T, Oka T, Nakane Y. Long-term results of living related kidney transplantation: A retrospective study on 114 recipients followed over 10 years. *Jpn J Surg.* 1991;21:138–144.
15. Inoue S, Horii M, Asano T, et al. Risk factors for nontraumatic osteonecrosis of the femoral head after renal transplantation. *Orthop Sci.* 2003;8:751–756.
16. Kubo T, Fujioka M, Yamazoe S, et al. Relationship between steroid dosage and osteonecrosis of the femoral head after renal transplantation as measured by magnetic resonance imaging. *Transplant Proc.* 1998;30:3039–3040.
17. Lebrun-Vignes B, Archer VC, Diquet B, et al. Effect of itraconazole on the pharmacokinetics of prednisolone and methylprednisolone and cortisol secretion in healthy subjects. *Br J Clin Pharmacol.* 2001;51:443–450.
18. Iwasaki K, Matsuda H, Nagase K, et al. Effects of twenty-three drugs on the metabolism of FK506 by human liver microsomes. *Res Commun Chem Pathol Pharmacol.* 1993;82:209–216.
19. Frey BM, Frey FJ. Clinical pharmacokinetics of prednisone and prednisolone. *Clin Pharmacokinet.* 1990;19:126–146.
20. Barth J, Damoiseaux M, Mollmann H, et al. Pharmacokinetics and pharmacodynamics of prednisolone after intravenous and oral administration. *Int J Clin Pharmacol Ther Toxicol.* 1992;30:317–324.
21. Gambertoglio JG, Amend WJ Jr, Benet LZ. Pharmacokinetics and bioavailability of prednisone and prednisolone in healthy volunteers and patients: A review. *J Pharmacokinet Biopharm.* 1980;8:1–52.
22. Grenier FC, Luczkiw J, Bergmann M, et al. A whole blood FK 506 assay for the IMx analyzer. *Transplant Proc.* 1991;23:2748–2749.
23. Rose JQ, Jusko WJ. Corticosteroid analysis in biological fluids by high-performance liquid chromatography. *J Chromatogr.* 1979;162:273–280.
24. Ono K. Diagnostic criteria, typing, and staging of avascular necrosis of the femoral head. *Annual Report of the Idiopathic Avascular Necrosis of the Femoral Head Ministry of Health and Welfare of Japan.* 1986:330–336.
25. Jusko WJ, Piekoszewski W, Klintmalm GB, et al. Pharmacokinetics of tacrolimus in liver transplant patients. *Clin Pharmacol Ther.* 1995;57:281–290.
26. Boswell GW, Bekersky I, Fay J, et al. Tacrolimus pharmacokinetics in BMT patients. *Bone Marrow Transplant.* 1998;21:23–28.

27. Anglicheau D, Legendre C, Thervet E. Pharmacogenetics in solid organ transplantation: Present knowledge and future perspectives. *Transplantation.* 2004;78:311–315.
28. Venkataramanan R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet.* 1995;29:404–430.
29. Ohzono K, Saito M, Takaoka K, et al. Natural history of nontraumatic avascular necrosis of the femoral head. *J Bone Joint Surg Br.* 1991;73:68–72.
30. Fujioka M, Kubo T, Nakamura F, et al. Initial changes of non-traumatic osteonecrosis of femoral head in fat suppression images: Bone marrow edema was not found before the appearance of band patterns. *Magn Reson Imaging.* 2001;19:985–991.

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